NITROGEN AVAILABILITY AND VEGETATIONAL COMMUNITY STRUCTURE ON A CLIFF SYSTEM IN THE SOUTHERN APPALACHIANS

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ERIC R. PURDY

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APPROVED BY:

Dr. Michael D. Madritch
Co-Chairperson, Thesis Committee

Dr. Gary L. Walker
Co-Chairperson, Thesis Committee

Dr. Howard S. Neufeld
Member, Thesis Committee

Dr. Sue L. Edwards
Chairperson, Department of Biology

Max C. Poole, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Abstract

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Eric Purdy
B.A., James Madison University
M.A., Appalachian State University

Thesis Co-Chairpersons: Gary L. Walker and Michael D. Madritch

Cliffs often harbor pristine and important ecosystems with environmental conditions that provide havens for rare species as well as function as repositories of high genetic diversity. While research in almost every ecosystem confirms the importance of nutrient cycling to community dynamics, it has been an under-investigated aspect of cliff ecology. This study explores the influence that nitrogen availability and other abiotic variables have on the diversity of vegetation communities on a cliff ecosystem in the southern Appalachian Mountains near Todd, NC, USA. Analysis of abiotic variables (slope, aspect, surface heterogeneity, soil cover, NO3-N, and NH4-N) partially confirmed observations from previous studies demonstrating that surface heterogeneity has a positive relationship and slope has a negative relationship ($r^2 = 0.38$, $p = 0.0009$ and $r^2 = 0.31$, $p = 0.0079$ respectively) with regard to total species diversity. Nitrogen availability along the cliff face increased with surface heterogeneity ($r^2 = 0.30$, $p = 0.0107$) and soil depth ($r^2 = 0.26$, $p = 0.0296$). In addition, total vegetational species diversity declined with increasing nitrogen availability ($r^2 = 0.63$, $p < 0.0001$). Competition and negative physiological effects observed

at high levels of nitrogen deposition are likely drivers of decreased plant diversity. This study is consistent with previous studies of cliff community response to basic abiotic variables and with studies that investigate the response of biota in non-cliff habitats to varying levels of nitrogen. Furthermore, the data presented in this study provide the first community level investigation of nitrogen cycling on a cliff and show that nitrogen availability is correlated both with existing abiotic variables and with the total vegetational species diversity of the studied cliff.

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Foreword

This thesis will be submitted to *Southeastern Naturalist*, a peer-reviewed journal owned and published by Eagle Hill publishing; it has been formatted according to the style guide for that journal.

Introduction

Background and threats to cliff systems

Research in the past two decades has demonstrated that cliffs have intrinsic biological and ecological importance, existing not just as barriers between ecosystems in a larger landscape but as refugia of biodiversity (Ursic et al. 1997, Larson et al. 2000a). Despite this recognition, cliff-face ecology is a relatively understudied field. The earliest studies of cliffs demonstrated that the absence of large-scale anthropogenic impact has resulted in the maintenance of remarkably undisturbed areas (Larson et al. 2000a). For instance, cliffs in the Niagara Escarpment in Ontario, Canada, are home to remnants of pre-colonial old-growth forests, and communities contained by cliffs in the Southern Appalachian Mountains are host to high levels of rare, disjunct, and endemic species (Clebsch and Walker 1988, Kelly et al. 1992, Kelly and Larson 1997, Smith 1998). The frequent occurrence of rare and disjunct species is facilitated by low levels of disturbance coupled with the unique abiotic conditions of cliff systems (Baskin and Baskin 1988). For instance, steep north-facing cliffs in Kentucky and Tennessee harbor boreal relict bog species (Walker 1987, Hill 2009, Larson et al. 2000a, b). Disjunct species such as *Thuja occidentalis* L. (Northern White Cedar) along with associated phanerogams in the Southern Appalachians are commonly found associated with cliff systems in this region (Caplenor and Spier 1975, Walker 1987, Clebsch and Walker 1988). Recent studies at Cumberland Gap National River and Recreation Area, on the border of Tennessee, Kentucky, and Virginia, confirm the consistent presence of rare vascular species associated with Southern

Appalachian cliffs (Ballinger 2007, Harkey 2013). The pristine nature of cliff systems, coupled with their rare microenvironments, give cliffs importance as havens for rare and disjunct species.

Cliffs, by virtue of their primarily vertical structure and inaccessibility, have been isolated from most negative anthropogenic impacts, but increases in atmospheric pollution and climate change could pose substantial threats to cliff communities. Large anthropogenic inputs of nitrogen (N) to the global cycle that have occurred in the past 150 years (Galloway et al. 2008) may have important consequences for biodiversity, especially in sensitive ecosystems such as cliffs. Independent studies have confirmed that lichens, which are the dominant species type on many cliffs, are particularly susceptible to excess N deposition, with increases in N deposition negatively correlated with lichen diversity (Johansson et al. 2011, Johansson et al. 2012, Stevens et al. 2012). Other communities are also negatively affected by excess N deposition, particularly wetlands and grasslands (De Schrijver et al. 2011, Fornara and Tilman 2012), while forested systems are less affected (De Schrijver et al. 2011). Cliff systems were not evaluated in this meta-analysis, but because they contain species known to be sensitive to elevated N deposition, they may also be at-risk (Kelly and Larson 1997, Smith 1998, Stevens et al. 2012).

Previous cliff studies

Substantial progress has been made toward understanding the trends and patterns found in the community structure of cliff systems (Larson et al. 2000b, Kuntz and Larson 2006b, Ballinger 2007, Hill 2009), but most studies have focused largely on the effect that abiotic conditions have on species abundance. The majority of cliff studies have either concentrated on a single species of interest or on a limited range of abiotic conditions such

as soil distribution, surface heterogeneity, moisture, disturbance, aspect, position, or light availability. Soil presence and volume are positively correlated with plant cover, and surface heterogeneity is positively correlated with vascular plant and lichen diversity (Smith 1998, Kuntz and Larson 2006b). Community assemblages on cliffs display vertical zonation, where the cliff edge community structure is distinct from the structure of communities found on the cliff face and the talus. In most cases, higher vascular diversity is found on the cliff edge and talus than on the cliff face (Ashton and Webb 1977, Larson et al. 2000b, Smith 1998, Ursic et al. 1997, Hill 2009).

Plant distributions are most affected by moisture and light availability. For example, bryophyte diversity increases with increased moisture. Moisture and light availability, controlled partially by aspect, often co-vary; and increased light and decreased moisture negatively correlate with vascular plant diversity (Oosting and Anderson 1937, Coates and Kirkpatrick 1992, Kelly and Larson 1997). A study in the Obed River Gorge, TN, demonstrated that variation in species composition is due mainly to small-scale microsite characteristics (Hill 2009). Previous studies of cliff abiotic conditions often generalized the abiotic conditions of entire cliff transects (Larson et al. 2000a, b), but Hill (2009) presented evidence that changes in abiotic conditions on a meter to meter basis strongly influence the biota present on a cliff, indicating that detailed sampling of biota and abiotic conditions needs to be conducted for an accurate measurement of abiotic variables and their influence.

Nutrient cycling

The inclusion of nutrient cycling in community studies has been common practice in a wide range of ecosystems from grasslands and tundra, to forests and wetlands (Bedford et al. 1999, Scherer-Lorenzen et al. 2003, Alberti et al. 2011). For instance, wetland

species diversity decreases with increasing N and phosphorus availability (Bedford et al. 1999). However, nutrient cycling and its effects have not yet been included in any comprehensive community analysis on cliffs. Nitrogen levels, and more broadly, nutrient limitation, have been studied in a limited capacity on cliffs, but they have been constrained in scope to the effect on a specific species of interest. Matthes-Sears and Larson (1990) investigated the effects of light, temperature, and moisture on the rate of carbon uptake in *Thuja occidentalis*, showing that carbon uptake was most often limited by light, while limitation by temperature or moisture was rare. Matthes-Sears et al. (1992) showed that macronutrient limitation or lack of mycorrhizal associations were unlikely to cause stunted growth of *T. occidentalis*, and further examination of *T. occidentalis* on cliffs determined that phosphorus is likely limiting production (Matthes-Sears and Larson 1995, Matthes-Sears et al. 1995). These studies made an effort to quantify the importance of nutrient availability to Northern White Cedar on cliff systems but did not assess the broader community structure (Matthes-Sears et al. 1995).

As lichens and bryophytes account for a substantial proportion of biodiversity on cliff systems and these lifeforms are highly dependent on both N and P for their growth and survival, it becomes important to study their response to nutrient availability in order to fully understand the constraints on nutrient cycling in these unique systems. While no studies have taken place on cliffs, several studies in laboratory and forest settings have focused on the importance of nutrient addition to lichen and bryophyte growth rates and the subsequent effect on community structure (Dahlman et al. 2004, De Schrijver et al. 2011, Johansson et al. 2012, Stevens et al. 2012). Johansson et al. (2012) explored the response of several epiphytic lichen species to increasing levels of N addition. *Alectoria sarmentosa*,

for example, was found to be highly sensitive to even low levels of N addition with decreased abundance in the sampled community. The majority of sampled lichens in the study exhibited a similar negative response to N deposition, but *Platismatia glauca* and *Hypogymnia phyodes* were found to be very N tolerant, with even large additions of N producing little physiological response. The removal of N-intolerant lichen species allowed N-tolerant species such as *Hypogynmia phyodes* to increase in abundance within the sampled community. The response to N additions observed in Johansson et al. (2012), where species of lichen showed differences in their N optima, can be seen in the results of other published studies. Vilsholm et al. (2009) reports that some lichen species respond positively to increases in N deposition, while Stevens et al. (2012) showed widespread decreases in lichen species richness with increased N deposition. Similar trends are seen with deposition of other nutrients, such as phosphorus (Benner and Vitousek, 2007).

Despite the importance of nutrient cycling in lichen and bryophyte communities, there have been no focused studies that target nutrient cycling or availability on cliff ecosystems. This distinction is particularly important as the physical structure of cliffs creates unique conditions that affect the processes involved in nutrient cycling. The vertical structure of cliffs prevents soil from accumulating, which, in turn, creates a system with little capacity to store nutrients that occurs in most other ecosystems. Storage of cliff nutrients is likely highly spatially heterogeneous, and what storage does occur most likely happens in small pockets of soil or organic matter that accumulate in crevices (Chapin et al. 2012). Furthermore, without soil to retain moisture, the rate of N mineralization and eventual availability to vegetation is likely to be lower than that found in more typical ecosystems (Knoepp and Swank 2002). Due to the vertical nature of most cliff systems,

nutrient additions in precipitation are likely to run off the cliff face and accumulate in the talus. As a result, any additions likely have a very short residence time on the cliff face, which differs substantially from what occurs in more horizontally oriented ecosystems (Larson et al. 2000b). Furthermore, the lack of soil, high moisture stress, and short residence times for nutrients may lead to the development of entirely different microbial communities, which in turn, may exert a large influence on the cycling and processing of nutrients, and in particular, N, on cliffs (Fierer and Schimel 2002, Chapin et al 2012).

This study expands on previous species-specific cliff nutrient research by taking a comprehensive community-level approach. It uses systematic nutrient sampling with a modified ion exchange method, coupled with analyses of abiotic factors and a survey of the biota present, to provide insight on plant community composition in cliff systems. This study aims to determine if there is a quantifiable relationship between levels of N availability measured on the cliff face and the biota present there. In addition, it seeks to analyze the relationship between important abiotic variables, such as surface heterogeneity and slope, and N availability on the cliff face, and to correlate such variation with community diversity and structural aspects.

Methods

Sampling procedure

Sampling was performed on an 18 m tall, 8 m wide undisturbed cliff, at the Gilley Biological Station in Todd, NC. The cliff is located at N 36º17.914' W 81º35.626' with an elevation of 958 m. Photographs are included in Appendix B. This cliff was chosen based on size and accessibility. The relatively small extent of the cliff allowed for a sampling protocol that encompassed the entire cliff area, and the location of the cliff ensured that the site was undisturbed by recreational rock climbing or hiking. The shape of the cliff face led to a sampling grid 6 m wide and between 11 and 15 m tall, with 1 m^2 plots arranged in a grid covering the cliff face. In total, seventy seven 1 m^2 plots were sampled. Within each plot, percent cover measurements were taken for each vascular plant, bryophyte, and lichen species. Samples of each species were taken from each plot for identification and for nutrient analysis.

Identification of non-crustose lichens was performed using Brodo and Sharnoff (2001), while Weakley (2006) was used to identify vascular plants. Bryophytes and crustose lichens were identified to morphospecies. In addition to biotic measurements, slope, aspect, surface heterogeneity, presence and absence of seeps, soil cover, and soil depth were measured. Surface heterogeneity assessment was based on a simplified version of the scale used in Kuntz and Larson (2006b), where the number of each type of feature (ledges, crevices, pockets) was recorded and multiplied by a weighted value corresponding to each feature type to obtain a measurement of surface heterogeneity. Resulting surface

heterogeneity values are unitless with higher values corresponding to rougher surfaces. Aspect measurements in previous studies were used as a macro-level variable to compare different cliff systems. However, in this study, there is a single cliff facing 278^{o} west, and as such large variations in aspect were not observed. Instead, aspect measurements were taken at each plot to determine if micro-scale variations in aspect were important. Aspect measurements, recorded for each plot, were converted from ordinal compass measurements into two linear variables. Northness comprised the north-south component (northness = cos(aspect)), and eastness comprised the east-west component (eastness = sin(aspect)). This transformation, previously used in Kuntz and Larson (2006b), allowed for the uniform treatment of aspect values in relation to the other physical factors.

Nutrient analysis

Ion resin nutrient traps were used to measure nitrate-N and ammonium-N availability along the cliff face. The traps were made from ion exchange resin sheets (Membranes International Inc, Ringwood, NJ). CMI-7000 and AMI-7001 membranes were used to absorb cations and anions, respectively. Once cut into 5x5 cm strips, cation and anion traps were attached to the cliff face using 3M™ double-sided foam outdoor mounting tape. Before field deployment, laboratory controls were analyzed which confirmed that N was not added to the traps by the mounting tape. The area of attachment on the cliff was cleaned with wire and fine bristle brushes in order to remove granular material that would inhibit adhesion. Traps were placed at the bottom center of each 1x1 m plot and collected after three months (May-July 2012). In addition, traps were placed on platforms under the forest canopy at the cliff edge and on platforms in an open field 1.3 km

away at the Gilley Biological Station to provide information on the relative levels of atmospheric deposition and throughfall.

Once collected, the traps were processed in the lab for NH_4^+ and NO_3^- using a KCl extraction and subsequent spectrophotometric analysis. Collected resin sheets were placed in scintillation vials after the portion containing the adhesive strip was removed. KCl (2M) was added to the vial, which was then shaken for 2 hours before the resin sheet was removed. This extract was then assayed for NH_4^+ and NO_3^-. In both assays, unknown samples were compared to a standard curve prepared to contain exact and known concentrations of NH_4^+ and NO_3^-. The absorbance of both known standards and unknown samples was read using a SpectraMAX 190 spectrophotometric plate reader (Molecular Devices, Sunnyvale, CA). Details on specific reagents and techniques used in both assays can be found in Madritch and Hunter (2002).

Once identified, vascular, bryophyte, and lichen materials were processed for total nitrogen (N). Samples were ground and then freeze-dried using a FreeZone freeze dryer (Labconoco, Kansas City, MO). After complete drying, a 15 mg sample was weighed into tins and analyzed using combustion analysis in a FlashEA NC 1112 Soil Analyzer (Thermo Fisher Scientific, Waltham, MD) to determine %N in each sample.

Statistical analysis

Species diversity of vascular plants, bryophytes, lichens, and total species were calculated for each plot using the Shannon-Weiner diversity Index (H). A nonmetric multidimensional scaling analysis (NMS) was performed for vascular, bryophyte, lichen, and total species groups using PC-ORD 6.0 (Peck 2010). This analysis used plots as sample units to assess the relationship between species percent cover measurements and abiotic

factors including nutrient levels and surface heterogeneity. The necessity of averaging surface heterogeneity for each plot obscured the small-scale relationship between lichen location and microtopographic features, so a qualitative analysis was performed using field notes and photographs.

Regression analyses were performed between abiotic factors and total species diversity to describe specific relationships between individual variables. Geographically weighted regressions (GWR) were used to determine relationships between measured abiotic variables and species diversity to account for non-independence among sample plots. GWR is a local analysis that is primarily used in geographic data to account for non-stationary and geographically non-independent data (Brunsdon et al. 1996, Fotheringham et al. 2002, Nogues-Gravo 2009, Kumar et al. 2012). While the data are stationary, this method is ideally suited to account for the non-independence in the sample plots. It uses a moving window to look at both data location and attributes. This approach allowed for a spatial analysis of the cliff system that accounts for the interaction between plots while allowing for a sampling resolution that can determine trends in N levels. A GWR was performed between each environmental factor and diversity measure using GWR 4.0 (Brundson 1996, Nakaya et al. 2012). An alpha level of 0.05 was chosen as the threshold for significance.

A partial least squares analysis (PLS) was run using SAS JMP 10 (SAS Institute, Cary, NC). This provided information on the importance of each abiotic factor measured by comparing their Variable Importance Plot (VIP) scores. Factors with a VIP > 0.8 were considered to contribute to the PLS model of total species diversity (Eriksson et al. 2006).

Data for each identified morphospecies were compared individually and for each major group: bryophyte, lichen, and vascular plants. A one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison tests was performed in SAS JMP 10 to determine differences in mean N between species groups. Homogeneity of variances was confirmed using Bartlett's test (Snedecor and Cochran 1989).

Spatial kriging analyses were performed to interpolate between the values of each variable, and these data were then used to create figures showing the spatial variation present. This allowed for visual exploration of the data and a means of qualitatively comparing variables (Figs. 1-3). Kriging analyses were performed on abiotic variables and diversity measures using the spatial analyst tools in ArcGIS 10 (ESRI, Redlands, CA).

Results

Abiotic and biotic variables

The study cliff had a mean slope of 86^{o} from horizontal, with a maximum of 120^{o} corresponding with overhanging cliff areas and a minimum of 36^{o} corresponding with ledges (Fig. 1a). Qualitative assessment of Figure 1a shows steeper slopes at the top of the cliff with a transition to more gradual slopes at the bottom. Surface heterogeneity measurements had a mean of 3.94, a maximum of 6.12, and a minimum of 0.5 (Fig. 1b). The macro-aspect of the cliff is 278^{o} and corresponds to the single west facing cliff. Micro-aspect measurements taken at each plot ranged from 261^{o} to 310.^{o}

Nitrogen inputs on the cliff face varied over small spatial scales (Fig. 2). Nitrate-N inputs were highest on the left and top left portions of the cliff, while ammonium-N inputs were highest on the bottom left of the cliff. Both nitrate-N and ammonium-N inputs were lower on the face and at the cliff edge under the canopy than in a nearby open field ($p < 0.0001$), but no significant difference existed between the cliff face and the edge (Table 1).

A total of 77 different taxa were identified to morphospecies: 10 bryophyte species, 24 vascular plant species, and 43 lichen species. The variation in Shannon-Weiner Diversity Index for total species, lichen, bryophytes and vascular groups is represented in Figure 3. Total diversity (H_{total}) was the greatest on the right and left side near the bottom of the cliff face (Fig. 3a). Lichen diversity (H_{lichen}) was the highest in the areas on the right side of the cliff (Fig. 3b). This corresponds to the highest areas of H_{total} in Figure 3a. Bryophyte diversity ($H_{bryophyte}$) shows a similar effect, where high levels in the lower left of

the cliff correspond to raised levels of H_{total} in Figure 3a. Figures 3d and 3a show less similarity. Crustose lichens were more common on smooth patches of rock, while fruticose and foliose were more common on rougher surfaces. Leprose lichens, such as the nearly ubiquitous *Lepraria sp.*, were commonly found in small crevices and under ledges. An analysis of microtopographic features performed by Kuntz and Larson (2006b) found similar results featuring specific species group responses to different rock features.

Multivariate analysis

NMS analysis showed no strong groupings for plot diversity. In addition, the influence of abiotic variables on plant community structure was low, as indicated by small vectors associated with the variables (Fig. 4). In addition to NMS, a PLS analysis was used to determine the relative importance of each measured abiotic variable to total species diversity. The variables with a VIP score greater than 0.8 were used to construct a model of predicted diversity. Modeled diversity was positively correlated to measured species diversity with $r = 0.52$ and $p < 0.0001$ (Fig. 5). The factors most important to the model of predicted diversity were slope, followed by surface heterogeneity, soil, and nitrogen (Table 2). Aspect was not included as a factor in the PLS model as it fell below the 0.8 threshold.

Geographically weighted regression (GWR)

The individual relationships between the abiotic factors and total species diversity were explored using linear regressions. Slope, surface heterogeneity, and nitrate-N levels were the most important factors associated with diversity (Table 3). Total species diversity decreased with increasing slope and nitrate levels, while diversity increased with increasing surface heterogeneity. While simple linear regressions provide insight to general trends, they are inadequate due to non-independence between the plots and must be complemented

with spatially explicit models. The sampling protocol used in this study was designed to elucidate the importance of small-scale variation in N levels; but unfortunately, placing plots in a grid introduces interaction and non-independence between plots. Global linear regressions were performed, but in order to compensate for the spatial interaction between adjacent plots, a geographically weighted regression was used to model the relationships between the abiotic factors and diversity. The geographically weighted regressions performed better than did the global linear regressions, as demonstrated by lower AICc values in each of the GWR models (Table 3). GWR analyses of individual diversity categories (lichen, bryophyte, and vascular diversity) were not feasible due to the scarcity of the data. Consequently, I report only GWR analysis of H_{total} (Table 3).

Nitrate-N levels were affected by several of the physical factors measured. Most significantly, nitrate levels increased with increasing surface heterogeneity and soil depth (Table 4). Additionally, the relationship between nitrate levels and micro-aspect was significant with eastness positively related to nitrate levels (Table 4). A negative regression between slope and nitrate-N levels was marginally significant (Table 4).

Lichen nitrogen content

Thallus N levels were measured for each lichen morphospecies collected (Fig. 6). The one morphospecies collected with a N-fixing photobiont, *Leptogium lichenoides,* showed a thallus N level of 5.7%, more than twice as high as the next highest morpohospecies, Bryophyte 5. However, only one individual of *Leptogium* was found, limiting its usefulness. Nonetheless, N levels found in this species are consistent with those found in N-fixing lichens in other studies (Dahlman et al. 2004, Johansson 2012). Thallus %N was significantly lower in lichens than in bryophytes or in vascular plants (Fig. 7).

Lepraria incana was widespread across the entire cliff face, and the thallus N levels in this species were compared to measurements of nitrate-N and ammonium-N collected using the ion-resin traps. The thallus N % in *Lepraria incana* did not vary spatially with any nitrate-N or ammonium-N levels and no significant geographically weighted regressions were found between *Lepraria incana*, thallus %N, and the measured abiotic variables.

Discussion

General abiotic variables

Small-scale variation in cliff-face vegetation communities has been the focus of several studies since the early 1990s. Most studies have addressed the same abiotic factors, and many have focused on the effects of disturbance, usually rock climbing, on vegetation community structure. When the physical factors most commonly considered in previous cliff community studies, slope, aspect, and surface roughness or heterogeneity, were measured in this study, the results were consistent with previously published research in the field (Matthes-Sears and Larson 1995; Kuntz and Larson 2006a, b; Adams and Zaniewski 2012). For instance, slope had a negative relationship with total species diversity (r^2 = 0.306, p = 0.0079), likely due to the space needed for propagule attachment and vascular plant establishment (McConnaughay and Bazzaz 1991, Matthes-Sears and Larson 1995, Matthes-Sears 1999). In addition, global linear regression suggests that bryophyte diversity was negatively related to cliff slope, again most likely due to conditions necessary for recruitment (Cutler et al. 2008, Garbotti et al. 2011). Slope is consistently an important predictive variable (Kuntz and Larson 2006b) and while not strongly significant in this study, is likely biologically significant.

Surface heterogeneity was positively related to total species diversity, similar to findings by Kuntz and Larson (2006b) and was one of the most important physical variables identified by the PLS model (Table 3). Bryophyte, lichen, and vascular diversity were each individually positively related with surface heterogeneity. This relationship is

also likely due to recruitment effects in bryophyte and vascular plants. For lichens, qualitative observations suggest that plots with varying rock features, and therefore high surface heterogeneity, host a greater variety of different lichen species and forms. For example, crustose lichens were more common on smooth patches of rock, while fruticose and foliose were more common on rougher surfaces. Leprose lichens, such as the nearly ubiquitous *Lepraria sp.*, were commonly found in small crevices and under ledges. However, the surface heterogeneity measurements, taken at the 1 m^2 plot size, were far too coarse to provide data suitable for a quantitative analysis of the effects of surface heterogeneity on individual lichen morphologies. Fine scale microtopgraphic measurements would likely resolve this issue.

Nitrogen availability

In an attempt to expand the scope of abiotic factors measured and their potential effects on community composition, N inputs were measured along the face of the cliff. The N sampling methodology developed for this study was originally attempted with the transect procedure commonly used in cliff ecology (Hill 2009, Kuntz and Larson 2006b, Smith 1998). In an initial survey of N on a cliff face, with plots placed every 3 m, no meaningful relationship was found between N levels and any measured physical or biotic variable (unpublished data). In addition, large N spikes were measured in isolated areas. In an attempt to explain patchy N dynamics seen in preliminary studies, the sampling protocol for this study was revised to use a much finer scale. Plots were placed every meter and the sampling was expanded laterally into a grid. Once data were collected on this finer resolution scale, relationships began to emerge as the large and idiosyncratic spikes became interpretable trends. Nitrate levels were found to be positively related with

surface heterogeneity, soil depth, and eastness (Table 4). Only at this finer scale did concrete relationships between variables measured in this study and accumulation of N on the sampled cliff face become clear.

Several possible N sources exist for cliff faces. First among them is atmospheric deposition, a source that could be influenced by several factors. Dry deposition in the form of impaction could be influenced by the roughness of the cliff face. Areas of high surface heterogeneity could be more efficient at catching dust or aerosols that would carry N into the system (Ruijgrok et al. 1997, Petroff et al. 2008), and it is likely that the positive regression between nitrate inputs and surface heterogeneity (Table 4) is at least partially due to this. However, within the bounds of this experiment, it is not possible to separate dry deposition from other sources of N. Wet deposition and runoff from the cliff edge likely also contribute to the pattern of N inputs on the cliff face, and this pattern is, in turn, likely influenced by the abiotic variables surface heterogeneity and soil depth. The cracks and shelves that contribute to high surface heterogeneity trap water and its nutrients, and the soil pockets that form in areas of high surface heterogeneity would be even more efficient at catching this water. Both direct wet deposition and leaching from N pools on the cliff edge likely contribute to the N content observed in these pockets. Furthermore, these large cracks and shelves are capable of catching litter that falls from the cliff edge and overhanging trees. The cracks and ledges large enough for soil to form exhibited a statistically significant positive relationship between soil depth and NO_3^- levels. It is possible that the N present in these soil pockets could then leach out to be trapped by pockets further down the cliff. Indeed, the general trend seen in ammonium-N levels indicates higher concentrations of N in plots lower on the cliff (Fig. 2a). One study of rock

outcrops in Brazil indicated that small isolated soil pockets forming on rock had extremely low cation exchange capacity (CEC) values (Benites et al. 2007). If the Gilley cliff exhibits a similar CEC this possibility could explain the distribution of NH_4^+ observed on this cliff and the ease with which NH_4^+ seems to leach down the cliff. However, lack of research investigating the internal nutrient cycling of cliffs makes it difficult to accurately discuss the causes and mechanisms behind the values of nitrogen observed on the cliff face, and as such it cannot be assumed that N content in these pockets is controlled solely by deposition.

The negative relationship between slope and nitrate-N levels is marginally significant ($r^2 = 0.218$, $p = 0.059$) but possibly biologically important and influenced by surface heterogeneity. Runoff, direct wet deposition, and litter accumulation would likely have a greater contribution to N levels on areas with gentler slopes. The regression observed between N availability and surface heterogeneity is likely a combination of wet and dry deposition deposited both directly by impaction and litterfall and indirectly by runoff.

The amount of nitrogen present in the thallus of lichens and the structure of vascular vegetation on the Gilley cliff differs according to taxa. Lichens had significantly less thallus %N content than did bryophytes or vascular plants (Fig. 7). This relationship was expected, as lichens lack the root systems of vascular plants that are involved in nutrient uptake (Dahlman et al. 2004). The one N-fixing species present, the lichen *L. lichenoides*, had thallus N levels more than twice as high as the next highest species (Fig. 6). On this cliff, only one individual of *L. lichenoides* was found, limiting the ability to make conclusions regarding N-fixing lichens, but studies by Johansson et al. (2012), and

Dahlman et al. (2004) support the assertion that N-fixing lichens contain large reservoirs of N and corroborate the %N content of the one species measured in this study. While the limited presence of N-fixers did not induce any measurable spike in N-levels, the large amount of N present in this species suggests that if a cliff has a high frequency of N-fixers, the nutrient dynamics and pools may be different than those on the cliff at Gilley Biological Station.

Nitrogen and community structure

Geographically-weighted regressions indicated that nitrate was negatively related with total species diversity ($r^2 = 0.62$, $p<0.0001$). The negative relationship between nitrate-N availability and species diversity is consistent with results found in other ecosystems. For instance, nitrate-N levels were negatively correlated with species diversity and richness in wetland and grassland systems (Bedford et al. 1999, Oelmann et al. 2011). N additions may be causing increases in competition and thus decreases in diversity and species richness (Huston 1997). In addition to competitive drivers such as the one described by Huston (1997), Johansson et al. (2012) suggests that, in the case of lichens, increases in N deposition may have consequential physiological responses that lead to lower diversity and richness. Johansson et al. (2012) showed that in multiple species of lichen, small additions of N were beneficial and increased growth rates. However, under the highest levels of N addition (50 kg N ha^{-1} y^{-1}), growth rates dropped below pre-addition growth rates. Johansson et al. (2012) concluded that N additions were positively correlated with diversity at low levels of N addition and negatively correlated at high levels of N addition. This context provides support for the decreases in diversity seen with higher

levels of N on the cliff system at Gilley. It seems likely that observed decreases in diversity are caused by a combination of negative physiological and competitive responses.

Forest, grassland, and wetland systems all have an extensive soil accumulation that influences above-ground and below-ground N interactions (Chapin et al. 1986). On cliffs, stores of N in soil pools do seem to exist, as supported by the positive regression between soil presence and nitrate levels (Table 3; however, the vast majority of the cliff surface is not covered by soil pockets that create these pools. Consequently, the sources of N available for cycling are likely more ephemeral, such as dry and wet atmospheric deposition and runoff. Nutrient levels, while largely unstudied on cliffs, have been studied in other rock outcrops and areas undergoing primary succession. Newly deglaciated areas and other areas of primary succession have large sources of available phosphorus from weathering rock and little accumulation of biomass to produce dead organic matter, soil, and associated N pools (Cutler et al. 2008, Cutler 2011, Garibotti et al. 2011). It falls to primary successional species such as lichens and bryophytes to weather the substrate, contribute to the initial accumulation of rock flour, fix and trap nutrients, and facilitate the transition to later successional stages when these systems are limited by phosphorus. Cliff systems share many similarities with these primary successional environments. Little to no soil is present, and they are dominated by early successional species. In newly-formed cliffs, the state of rock weathering would suggest that established communities are N limited (Adamo and Violante 2000). This, in turn, would lead to communities with low competition and high diversity (Huston 1997). However, old cliffs may harbor microenvironments with varying nutrient availability. Matthes-Sears et al. (1995) indicate that cliffs on the Ontario Escarpment may be phosphorus limited as *Thuja occidentalis*

growing there were found to be limited by that nutrient, but the proximity of the Niagra Escarpment to high-population areas may mean that N deposition in that area is high, which in turn could lead to decreases in lichen diversity and phosphorus limitation (Johansson et al. 2012). It is likely that N cycling on cliffs may be more similar to that in primary successional communities, such as those without large established soil pools, than they are with later successional environments such as forest or grassland ecosystems. Consequently, cliffs may represent steady-state primary successional communities. While primary successional communities in deglaciated areas eventually facilitate the accumulation of soil to bring about later successional stages (Garibotti et al. 2011), the limits imposed by steep slopes in cliff systems prevent any significant soil formation. The limitations imposed by limited soil reserves, such as rooting requirements and nutrient pools, limits the plant community to that of a permanent primary successional state.

This fine-scale study at Gilley Biological Station indicates that the link between plant diversity and N levels is consistent with other ecosystems. However, it is impossible to resolve conclusively the question of nutrient limitation without fertilization studies (Matthes-Sears et al. 1995, Johansson et al. 2012). Nitrogen additions at Gilley Biological Station should be performed, potentially with stable isotope N additions, in order to resolve the question of nutrient limitation. In addition, the nutrient sampling regimen should be expanded to include phosphorus to provide a comprehensive view of nutrient cycling and limitation on cliffs. Biomass measurements should be taken to assess the total N present in the area of the cliff as well. Furthermore, no research has yet addressed the soil chemistry or microbial communities found in cliff soil pockets. Neither has any research addressed rates of nitrogen transformations such as denitrification, nitrification, mineralization, or

ammonification. This study starts to investigate the influence of N on community structure, but much needs to be done in order to understand the mechanistic causes underlying the internal and external nitrogen cycle of cliffs.

It is of paramount importance that cliff systems are studied in further detail as N deposition and rock climbing may pose risks to the diversity of these systems. Direct anthropogenic disturbance, usually from rock climbing, has distinct and quantifiable effects on community composition (Kelly and Larson 1997, Smith 1998). Studies of climbing impacts on plant structure have demonstrated that foliose lichens, fruticose lichens, bryophytes, and vascular plants decrease in species abundance and diversity, while crustose lichens increase in diversity and abundance (Nuzzo 1996; Kelly and Larson 1997; Camp and Knight 1998; McMillan and Larson 2002). Indirect anthropogenic disturbance in the form of N-deposition will increasingly be a problem as population growth continues, and lichen communities will be among the first impacted (Stevens et al. 2012). Diversity of lichens is likely to decrease over time, especially around urban and agricultural areas. As such, the exploration of N cycling in cliff ecosystems will continue to be an important field of study, both to preserve the biodiversity sheltered by the abiotic conditions of cliffs, and to protect lichens, which can serve as indicators for excess N-deposition.

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Table 1. N input to cliff edge, face, and nearby field. Values are mean ± se, n = 80. Means comparison performed separately for ammonium-N and nitrate-N. Means followed by different letters are statistically different at $p < 0.05$ as determined by Tukey's test.

Form of Nitrogen	Edge	Field	Cliff Face
µg NH_4-N m^{-2} $month^{-1}$	132 ± 27 **a**	399 ± 222 **b**	129 ± 142 **a**
µg NO_3-N m^{-2} $month^{-1}$	64 ± 8 **a**	208 ± 37 **b**	125 ± 37 **a**

Table 2. Partial Least Squares (PLS) variable importance plot (VIP). Variables with a VIP > 0.8 contribute to the PLS model of total species diversity (Eriksson 2006). Higher numbers indicate variables are more influential in the creation of the model.

Variable	VIP
Slope (degrees)	1.2809
Northness	0.3797
Eastness	0.7896
Surface Heterogeneity	1.5214
Soil Cover (%)	0.8674
Soil Depth (cm)	1.0684
µg NH_4-N/m^2	0.8580
µg NO_3-N/m^2	0.8045

Table 3: Regressions between abiotic factors and total species diversity. The strength of the Global Linear Regression (GLR) and Geographically Weighted Regression (GWR) models are evaluated using the Corrected Akaike Information Criteria (AICc). Lower AICc numbers indicate a better fitting model. Bolding indicates regression with p-values < 0.05

Abiotic Factor	Slope	GLR r^2	GLR AICc	p-value	GWR r^2	GWR AICc	p-value
Slope	**(-)**	0.1374	392.3	0.2428	**0.3065**	386.1	**0.0079**
Eastness	**(-)**	0.006	403.3	0.9593	0.1479	397.3	0.2086
Northness	**(-)**	0.0113	402.8	0.9233	0.0708	402.9	0.5487
Surface Heterogeneity	**(+)**	0.0566	399.2	0.6322	**0.37715**	386.5	**0.0009**
Soil Cover	**(+)**	0.0093	402.9	0.9371	0.0562	402.2	0.6345
Soil Depth	**(+)**	0.0689	398.1	0.5595	0.18046	394.5	0.124
NO3-N	**(-)**	**0.2570**	380.8	**0.0327**	**0.6266**	346.7	**<0.0001**
NH_4-N	**(-)**	0.001	403.6	0.9935	0.1482	399.6	0.2078

Table 4: Regressions between µg NO_3-N m^{-2} $month^{-1}$ and abiotic variables. The strength of the Global Linear Regression (GLR) and Geographically Weighted Regression (GWR) models are evaluated using the Corrected Akaike Information Criteria (AICc). Lower AICc numbers indicate a better fitting model. Bolding indicates regressions with p-values < 0.05.

Abiotic Factor	Slope	GLR r^2	GLR AICc	p-value	GWR r^2	GWR AICc	p-value
Slope	**(-)**	0.0031	996.7	0.9797	0.2179	990.9	0.0700
Eastness	**(+)**	0.0231	995.1	0.8495	**0.2493**	989.3	**0.0374**
Northness	**(-)**	0.0127	995.9	0.9169	0.2342	989.7	0.0510
Surface Heterogeneity	**(+)**	0.0507	992.9	0.6768	**0.3034**	982.6	**0.0107**
Soil Cover (%)	**(+)**	0.0025	996.7	0.9836	0.1982	991.7	0.1000
Soil Depth (cm)	**(+)**	0.0062	996.5	0.9594	**0.2602**	986.4	**0.0296**

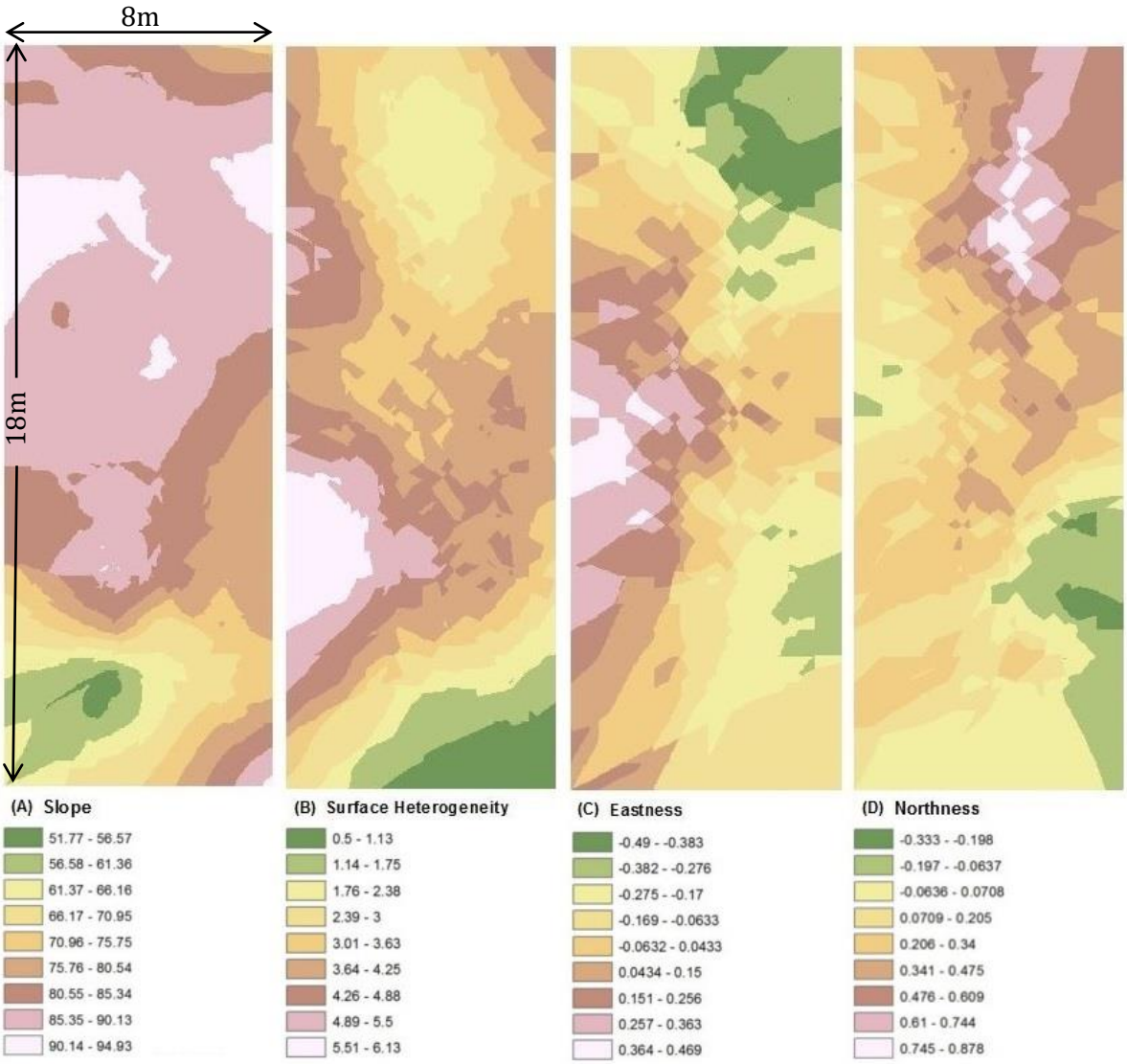

Figure 1. Spatial kriging of abiotic factors measured for each plot. Eastness and northness were calculated from ordinal aspect measurements. Each diagram is a scale representation of the 8m x 18m cliff face.

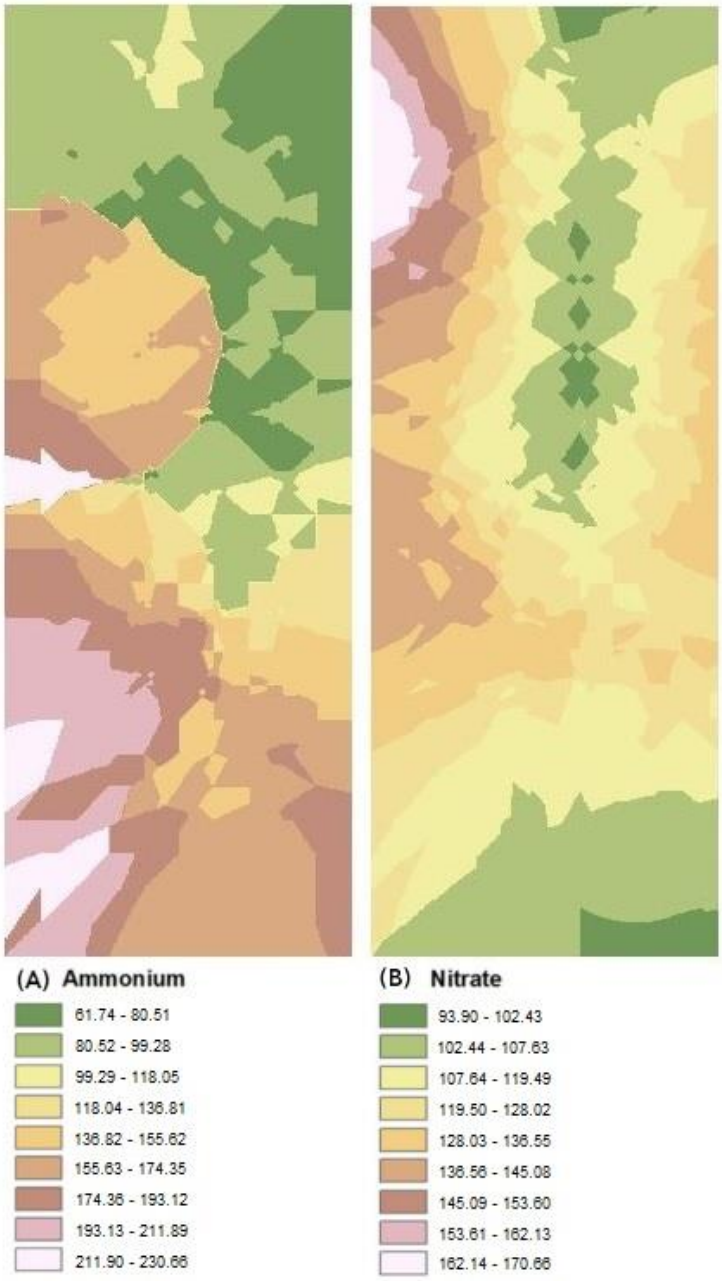

Figure 2. Spatial kriging of nitrate-N and ammonium-N on the vertical cliff face. Concentrations are µg NO_3-N m^{-2} $month^{-1}$ and µg NH_4-N m^{-2} $month^{-1}$.

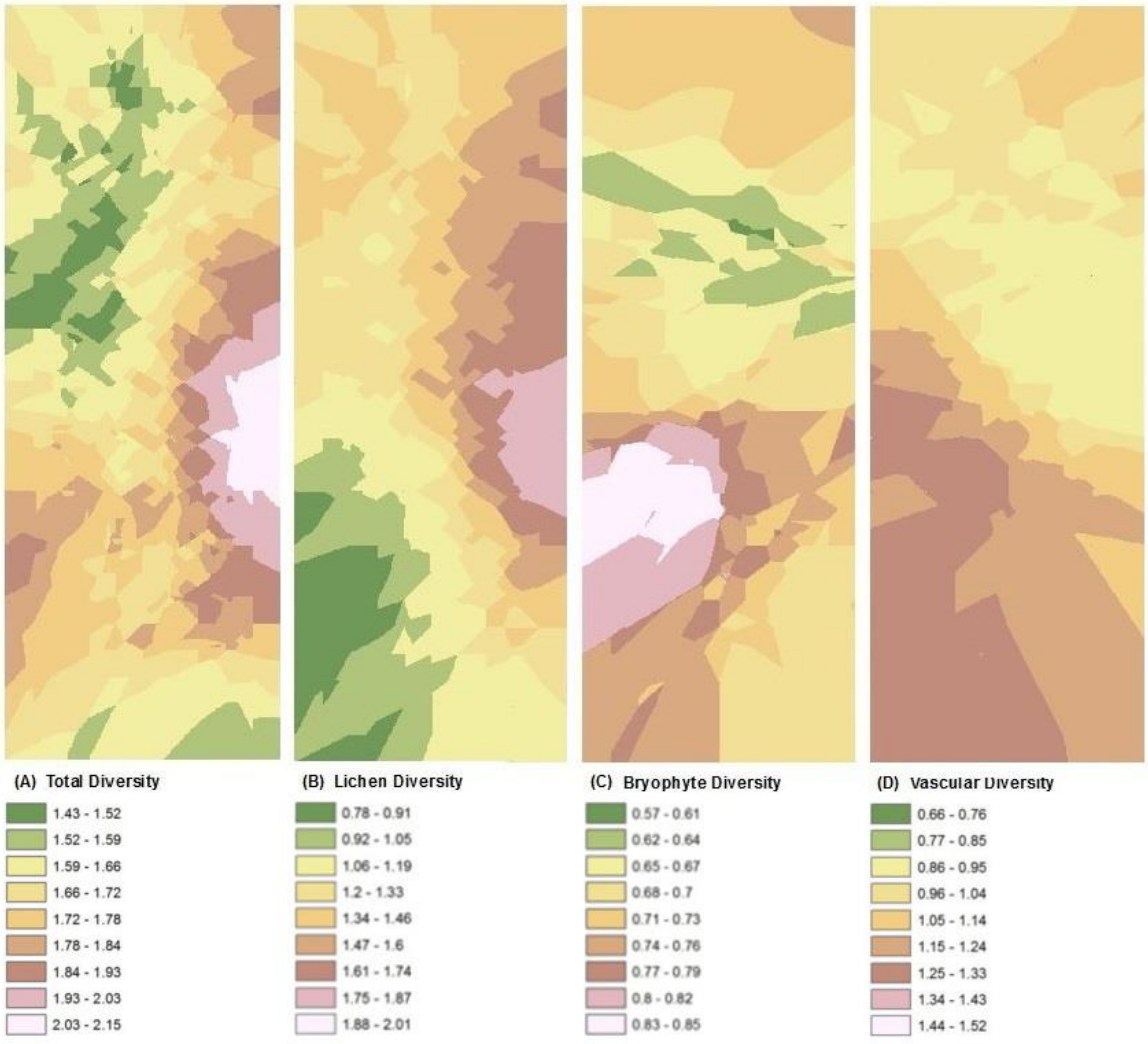

Figure 3. Spatial kriging of the Shannon-Wiener Diversity Index values for lichen, bryophyte, vascular, and combined (Total) species diversity on the vertical cliff face.

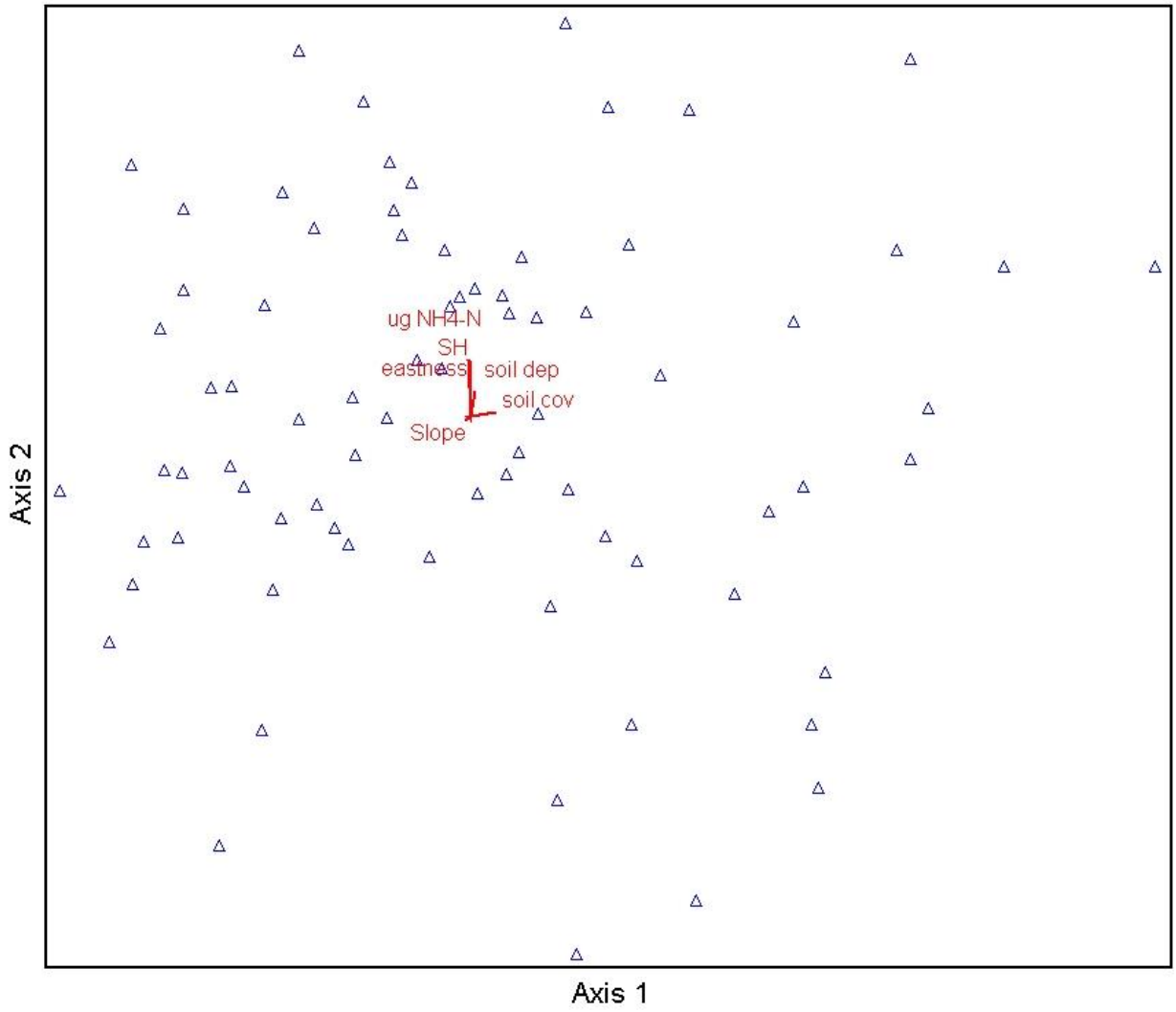

Figure 4. Non-metric multidimensional scaling (NMS) ordination biplot showing relationship among total species diversity in plots and abiotic variables. Blue triangles represent plots and the red lines represent the abiotic variables measured. The lengths of the vector lines correspond to importance of that variable to variation in plant diversity. SH: Surface Heterogeneity, ugNH4-N:µg NH_4-N m^{-2} $month^{-1}$, soil cov: % Soil Cover, soil dep: Soil Depth (cm).

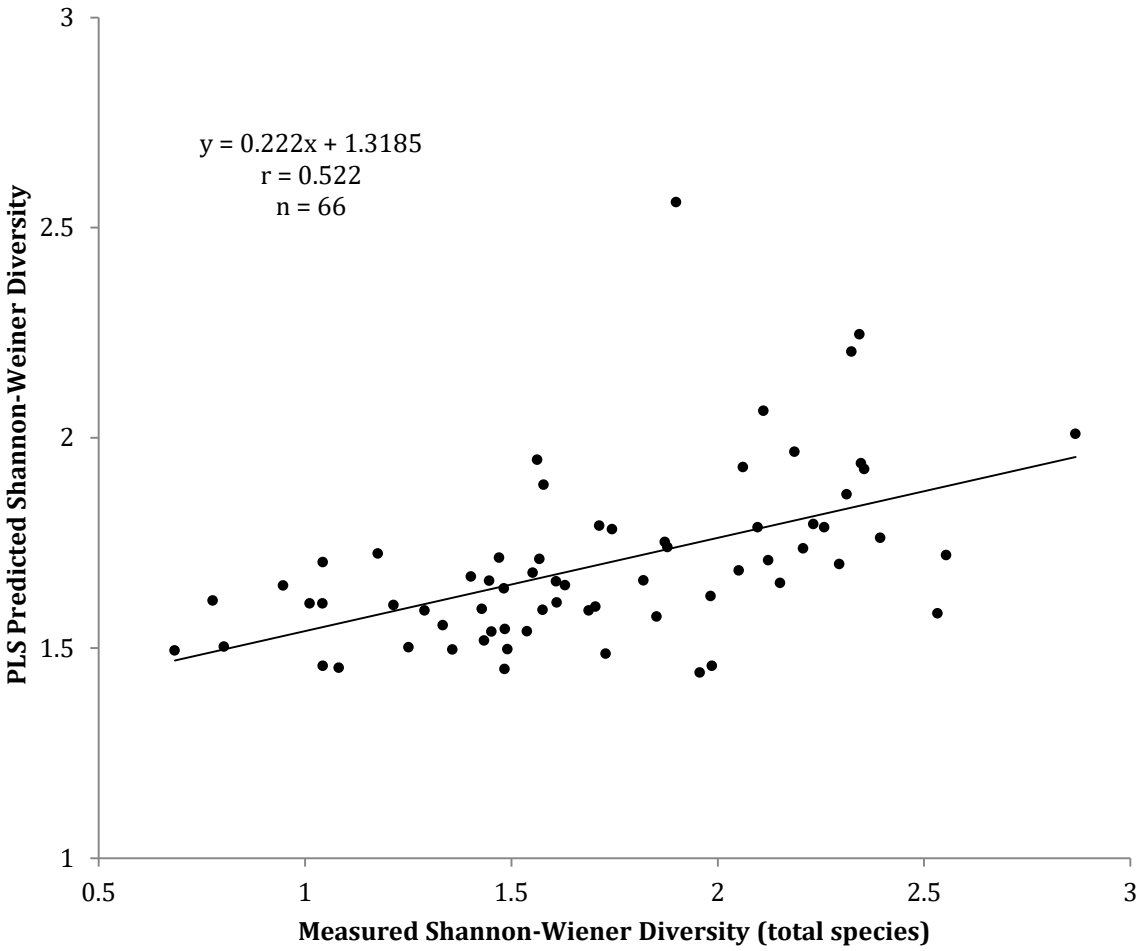

Figure 5. Regression between measured total species diversity and diversity as predicted by the Partial Least Squares model. $p < 0.0001$.

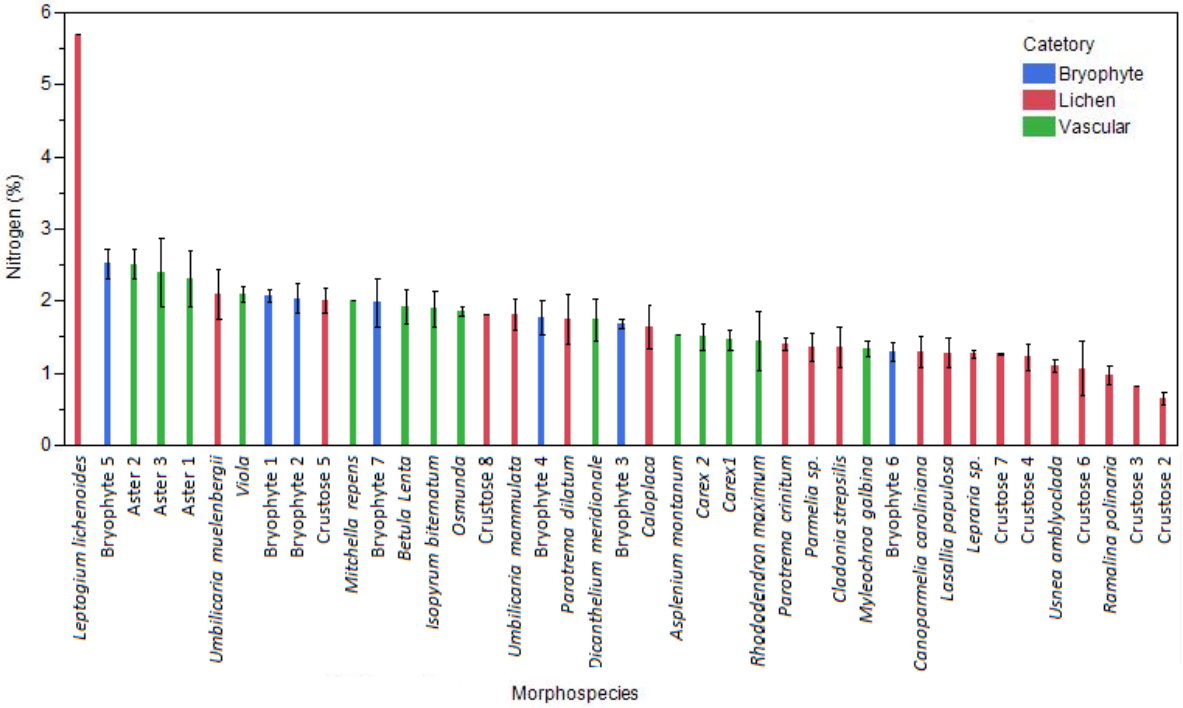

Figure 6. Mean thallus nitrogen for each morphospecies. Colors are grouped by species category type. *Leptogium lichenoides* was the only species that had a nitrogen fixing photobiont. Bars show mean ± standard error. n = 42.

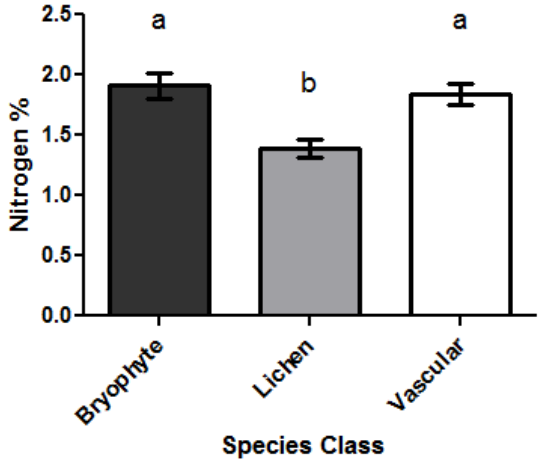

Figure 7. Thallus nitrogen for each species class. Lichens had lower nitrogen compared to either bryophyte or vascular groups. n = 42 Bars indicate mean ± se.

Appendix A

Species List

Vascular Plants

Asplenium montanum
Aster 1
Aster 2
Aster 3
Betula lenta
Carex 1
Carex 2
Dicanthelium meridionale
Isopyrum biternatum

Lichens

Caloplaca sp.
Canoparmelia caroliniana
Cladonia strepsilis
Crustose 2
Crustose 3
Crustose 4
Crustose 5
Crustose 6
Crustose 7
Crustose 8
Lasallia papulosa
Lepraria sp.
Leptogium lichenoides

Bryophytes

Bryophyte 1
Bryophyte 2
Bryophyte 3
Bryophyte 4
Bryophyte 5
Bryophyte 6

Bryophyte 7

Mitchella repens
Myleochroa galbina
Osmunda cinnamomea
Parmelia sp.
Parotrema crinitum
Parotrema dilatum
Ramalina polinaria
Rhododendron maximum
Umbilicaria mammulata
Umbilicaria muelenbergii
Usnea amblyoclada
Viola sp.

Appendix B

Photographs of the cliff study area

Photograph 1, View of cliff from talus. Photo looks up toward the cliff edge.

Photograph 2, View of Cliff from talus. Photo looks south along the west facing cliff.

Vita

Eric Purdy was born in Port Jefferson, New York. He and his three siblings were raised in New York and then Charlottesville, VA, by his parents Stephen and Cheryl Purdy. Upon graduating from Western Albemarle High School in 2006, Eric attended James Madison University where he graduated with a B.S. in Biology in 2010. After college, Eric was granted the opportunity to study cliff ecology at Appalachian State University where he graduated with a M.S. in Biology in 2015.